# Doctoral Thesis

# Analyses, Dynamics and Fates of Reactive Oxygen Species (ROS) in Natural Waters: Emphasis on Photochemical Reactions

## (Summary)

**Adebanjo Jacob ANIFOWOSE**

**Graduate School of Biosphere Science**

**Hiroshima University**

**September 2016**

別紙４

# 学 位 論 文 の 要 旨

論文題目　Analyses, Dynamics and Fates of Reactive Oxygen Species (ROS) in Natural Waters: Emphasis on Photochemical Reactions.

(天然水中の活性酸素種の測定、動態、運命――特に光化学反応に関して)

広島大学大学院生物圏科学研究科
環境循環系制御学専攻
学生番号　D130284
氏　　名　Adebanjo Jacob ANIFOWOSE

A brief introduction of the reactive oxygen species (ROS) – hydrogen peroxide ($H_2O_2$), hydroxyl radical (•OH), nitric oxide (NO•) and superoxide radical ($O_2^{\bullet -}$) – measured in some natural waters in this study was reported in chapter 1. $H_2O_2$ and $O_2^{\bullet -}$ mediate in redox chemistry of metals (Fe, Cu, Mn) and other chemical species in natural waters, thus making them important targets of many environmental studies. Moreover, $O_2^{\bullet -}$ is a precursor of $H_2O_2$. Similarly, •OH is a potent oxidizing agent capable of degrading recalcitrant organic pollutants in natural waters. NO• is photoformed in natural waters containing nitrite and it could be a potential sink of $O_2^{\bullet -}$ due to their high reaction rate. The reaction of oxygen with photo-irradiated dissolved organic matter (DOM) in natural waters produces $O_2^{\bullet -}$. However, measurement of this important ROS ($O_2^{\bullet -}$) has been a perennial challenge due to lack of suitable analytical technique. This and other environmental issues relating to the ROS in natural waters were addressed in this study.

Chapter 2 presents a report on the monthly NO• measurements in the Kurose River at Higashi-Hiroshima city. Results show that NO• photoformation rate ranged from 0.01 to 35.4 ($\times 10^{-10}$ M $s^{-1}$) with steady-state concentrations in the range of 0.02–68.5 ($\times 10^{-11}$ M). There was a strong correlation ($r^2 = 0.95$) between NO• photoformation rate and the nitrite concentration in the river showing $NO_2^-$ as a major NO• precursor. On the average, 98% of the photoformed NO• came from river nitrite. The NO• lifetime ranged from 0.05 to 1.3 s in the river and remained fairly stable in the upstream and downstream. The •OH radical, which was quantified during the study, had a photoformation rate of 0.01 to 13.4 ($\times 10^{-10}$ M $s^{-1}$) and a steady-state concentration of 0.04 to 119 ($\times 10^{-16}$ M) with a lifetime that ranged from 0.3 to 23 μs. •OH only accounted for ≤ 0.0011% of the total NO• scavenged, showing that it was not a major sink for river NO•.

In chapter 3, monthly measurements of $H_2O_2$ and •OH in rainwater and the Kurose River were carried out in 2013. $H_2O_2$ concentrations in the rainwater and river were highly season-dependent. $H_2O_2$ concentrations in the rainwater varied from the lowest 0.03 μM in winter to the highest 14.3 μM in spring. In the year, estimated wet deposition of $H_2O_2$ in Higashi-Hiroshima city was 7.5 mmol $m^{-2}$ $y^{-1}$. In the Kurose River, $H_2O_2$ concentrations of 0.06–0.37 μM were measured. The lowest and highest concentrations were found in the winter and summer, respectively. There was good correlation between solar intensity and $H_2O_2$ concentrations measured in the rainwater ($r = 0.79$, $p < 0.01$) and the river ($r = 0.81$, $p < 0.01$), which indicate photoproduction as one of the major $H_2O_2$ sources in the natural waters. The •OH was 1 order of magnitude photoformed in the river (~$10^{-10}$ M $s^{-1}$) than in the rainwater. $H_2O_2$ and the unknown (which probably include photo-Fenton reaction) were predominantly •OH photochemical sources in the rainwater, accounting for 0.2–48%, 43–84%, respectively. In contrast, $NO_2^-$ was the major •OH source (49–80%) in the Kurose River, while $H_2O_2$ contribution was negligible (<1%).

In chapter 4, daytime flux of NO• at the Seto Inland Sea-atmosphere boundary was measured in

September, 2013 and June, 2014. The average daytime $NO^{\cdot}$ concentration measured in the sea surface was $1.9 \times 10^{-11}$ mol $L^{-1}$ (~$9.87 \times 10^{-9}$ atm), while that measured over the sea was $5.2 \times 10^{-10}$ atm. Using an average wind speed of 3.2 m $s^{-1}$ measured over the Seto Inland Sea during the cruise, a daytime $NO^{\cdot}$ efflux of 0.22 pmol $m^{-2}$ $s^{-1}$ from the sea to the atmosphere was estimated. Assuming 8 h of solar intensity per day, about $1.54 \times 10^{6}$ g NO $y^{-1}$ efflux of $NO^{\cdot}$ from the 23,000 $km^2$ Seto inland Sea surface was determined.

In chapter 5, a method was developed for the measurement of $O_2^{\cdot-}$ in sunlit seawater using a fluorogenic probe – 3′,6′-(diphenylphosphinyl)fluorescein (PF-1). Reaction of the photoformed $O_2^{\cdot-}$ with PF-1 produced fluorescein, which was separated by isocratic HPLC and measured using a fluorescence detector at 490/513 nm (excitation/emission wavelength). The reaction rate constant of the probe with $O_2^{\cdot-}$ was pH-dependent: $(3.2–23.5) \times 10^{7}$ $M^{-1}$ $s^{-1}$ at $pH_{TOT}$ 7.65–8.50. The detection limit of $O_2^{\cdot-}$ photoformation rate was 1.78 pM $s^{-1}$. The method produced results that were consistent with those obtained in literatures when applied to ten (10) seawater samples from the Seto Inland Sea. The $O_2^{\cdot-}$ photoformation rates were 3.1–8.5 nM $s^{-1}$, with steady-state concentrations ranging $(0.06–0.3) \times 10^{-10}$ M. The method requires no technical sample preparation and can analyze large number of samples.

In chapter 6, $O_2^{\cdot-}$, $^{\cdot}OH$, $NO^{\cdot}$, and $H_2O_2$, in surface seawater obtained from the Seto Inland Sea were determined by concerted photochemical measurements. $O_2^{\cdot-}$ was photoformed at rates (range of $10^{-10}$ M $s^{-1}$) 1–2 orders of magnitude higher than $^{\cdot}OH$ and $NO^{\cdot}$. About 27% of the $O_2^{\cdot-}$ was transformed to $H_2O_2$. About 3% of the $O_2^{\cdot-}$ photoformed was consumed by $NO^{\cdot}$, while 15–21% of the $NO^{\cdot}$ was consumed by the $O_2^{\cdot-}$. Therefore, $O_2^{\cdot-}$ could be a major sink for $NO^{\cdot}$ in the sea. Estimated consumptions of $O_2^{\cdot-}$ by the $^{\cdot}OH$ and due to bimolecular dismutation were negligible. In the seawater, (+)-catechin- and humic-like fluorescent dissolved organic matter contributed 3.2–4.1% and 3.6–4.8% of the $O_2^{\cdot-}$ photoformation rates, respectively.

Chapter 7 gives a general discussion and major conclusions of this study. The four ROS in this study are ubiquitous in sunlit natural waters. Nitrite is a major photochemical source of $NO^{\cdot}$ and $^{\cdot}OH$ in natural waters. The $O_2^{\cdot-}$ concentration (~$10^{-11}$ M) in the Seto Inland Sea would be sufficient for speciation of the resident metals and other redox-active compounds.